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Toxicological characterization of ten medicinal plants used in the endogenous treatment of diarrhea

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Abstract:

This study aimed to explore the phytochemical and toxicological characteristics of ten 10 plants used in traditional treatment of infectious diarrhea in Benin. The acute toxicity of aqueous and hydro-ethanolic extracts of the plants was evaluated following the OECD 423 protocol at a single dose of 2000 mg/kg. This safety test was complemented by a larval cytotoxicity test. Hematological and biochemical examinations as well as a histological study on the liver and kidneys were performed. Larval cytotoxicity was assessed by the sensitivity of *Artemia salina* larvae to different concentrations of the plant extracts studied. The detection tests of chemical compounds were carried out according basis of differential staining and precipitation reactions. The mean lethal concentration (LC50) was determined by the probit method. The qualitative phytochemical screening of the plants studied revealed the presence of many chemical substances. This composition varied according to the plants studied. Acute toxicity data indicated that there was no mortality and no structural and functional alterations of the liver and kidneys of treated animals. Larval cytotoxicity data suggest that the plants studied were not cytotoxic. These observations reflect the safety of these plants and justify their use in traditional medicine in the treatment of many diseases including diarrheal diseases.

Keywords: Acute toxicity, Medicinal plants, Diarrheal diseases, Benin.



Introduction

Traditional medicine

- Based on the use of medicinal plants
- First reflex of more than 80% of the world's population for primary health care
- **In Benin:** this widely accepted trend is an ancestral medical practice that is transmitted from generation to generation



Introduction

Traditional medicine

- African pharmacopoeia Plants: Directed used against several diseases, particularly infectious ones
- Infectious Diseases Prevalence: High level in developing countries
- **Diarrheal diseases:** Deadliest infectious diseases, particularly among children and especially in West Africa.



Introduction

Diarrheal diseases

- Responsible for 1.8 million deaths each year worldwide
- 90% of which are among children under five years of age living in developing countries
- **In Benin:** diarrheal diseases are one of the main causes of morbidity and the pathogens are mainly **Bacteria**.



Introduction

Diarrheal diseases Traitment

- Difficult access to antibiotics by populations
- Antimicrobial Resistance
- Use of Medicinal Plants
- Ethnobotanical studies in West Africa: Provided information on the richness of African flora in the traditional management of diarrheal diseases



Introduction

Benin: West Africa Country

- Many Medicinal plants are used to treat diarrheal diseases
- But, very few scientific studies exist at this stage on the safety of anti-diarrheal plants
- This study was initiated to produce recent data on the toxicological characteristics of selected plants as used in traditional medicine.



Objective

Evaluate the larval cytotoxicity and acute toxicity of aqueous and hydro-ethanolic extracts of the selected plants on Wistar albino rats to predict their safety in the human species.



Material and Methods

Vegetal Material

Scientific Name	Botanical Family	Used Part
Anacardium occidentale	Anacardiaceae	Leaves
Daniellia oliveri	Leguminosae	Leaves
Diospyros mespiliformis	Ebenaceae	Leaves
Khaya senegalensis	Meliaceae	Bark
Manihot esculenta	Euphorbiaceae	Leaves
Occimum gratissimum	Lamiaceae	Leaves
Pterocarpus erinaceus	Euphorbiaceae	Leaves
Rauvoflia vomitoria	Apocynacea	Leaves
Senna italica	Leguminosae	Leaves
Vernonia amygdalina	Asteraceae	Leaves



Material and Methods

Animal Material

- Eggs of *Artemia salina* (ARTEMIO JBL D-67141 Gmbh Neuhofem) used for larval cytotoxicity test of selected medicinal plants.
- Wistar albino rats weighing between 130-180g used for acute toxicity testing

Reagents and consumables for chemical characterization



Material and Methods

Methods

Production of extracts

- fifty (50) grams of powder were macerated in 500 mL of solvent (water and water-ethanol).

Phytochemical analysis

- Detection of chemical groups were carried out according the method described by Houghton and Raman on the (1998) basis of differential staining and precipitation reactions.



Material and Methods

Methods

Cytotoxic effect

- Cytotoxic effect evaluated on larvae in the *Artemia salina* model according to method described by Dougnon et al. (2013)
- The LC50s obtained have been interpreted according to the standard established by Moshi et al. (2004)



Material and Methods

Methods

Acute toxicity

- Realized according the method described in OECD guideline 423
- Oral administration with a single dose of 2000 mg/kg body weight
- Duration: 14 days



Material and Methods

Methods

Hematological and Biochemical Examinations

- Hematological examination is NFS and Biochemical examinations were urea, creatinine, ASAT and ALAT

Histological Examinations: Carry out on the liver and kidneys

Statistical Analysis: SPSS 26.0



RESULTS AND DISCUSSION: Phytochemical Screening

Secondary metabolites	Test Reagent's	AO	DM	DO	KS	ME	OG	PE	RV	SI	VA
Tannins	Ferric chloride	+	+	+	+	+	+	++	+	++	++
Cathechic tannins	Stiasny's reagent	+	+	+	+	+	+	++	-	++	-
Gallic tannins	Ferric chloride and saturation with sodium acetate	+	+	-	-	+	+	++	-	-	++
Flavonoids	Shinoda test with powder Magnesium	-	+	+	+	-	+	++	+	-	++
Anthocyanins	Hydrochloric acid and ammonia at 50%	-	-	-	-	-	+	-	-	-	++
Leuco-anthocyanins	Hydrochloric acid	+	+	+	+	-	-	-	-	-	-
Alkaloids	Mayer's reagent	-	+	+	+	-	+	-	-	-	++
Mucilage	Absolute alcohol Test	-	-	+	+	-	-	-	+	++	++
Reducing compounds	Test with Fehling liqueur	+	+	+	+	-	+	+	+	+	+
Sterol-terpenes	Anhydride acetic-sulfuric acid	-	-	-	-	-	-	++	+	-	++
Saponosides	Foam index Test	+	+	+	+	+	+	-	+	+	+

+ : presence ; - : absence ; ++ : strong presence

AO : Anacardium occidentale ; DM : Dyospiros mespiliformis ; DO : Daniella oliveri ; KS : Khaya senegalensis ; ME : Manihot esculenta ; OG : Ocimum gratissimum ; PE : Pterocarpus erinaceus ; RV: Rauvoflia vomitoria ; SI: Senna italica ; VA: Vernonia amygdalina ;

Table 2: Qualitative phytochemical screening of the studied medicinal plants

Tannins are identified in all the plants studied

This presence and flavonoids justify the medicinal properties of the plants studied and their therapeutic uses in several pharmacopoeias

Similar observations are reported in the literature (Ojo, et al., 2013; Ajayi et al., 2017; and Nkoua Badzi et al., 2018).



RESULTS AND DISCUSSION:

Larval cytotoxicity

Plants	CL ₅₀ (mg/ml)	R ²
<i>Anacardium occidentale</i>	0.65	0.93
<i>Daniella oliveri</i>	0.04	0.64
<i>Dyospiros mespiliformis</i>	1.93	0.91
<i>Khaya senegalensis</i>	1.06	0.95
<i>Manihot esculenta</i>	0.52	0.92
<i>Ocimum gratissimum</i>	0.1	0.75
<i>Pterocapus erinaceus</i>	0.15	0.73
<i>Rauvoflia vomitoria</i>	0.56	0.83
<i>Senna italica</i>	0.02	0.92
<i>Vernonia amygdalina</i>	0.43	0.96

According the norm establish by Moshi 2004, the plants are not cytotoxic excep *Senna italica* and *Daniella oliveri*

Similar observations were reported by Dehou et al. (2018) and Déguénon et al. (2018) for *Ocimum gratissimum* and Soha et al. (2019) for *Khaya senegalensis*

Table 3: LC₅₀ of the studied medicinal plants and their interpretation



RESULTS AND DISCUSSION:

Acute Toxicity

- **LD₅₀ of the studied plant extracts**

No mortality was noted in the animals of the different lots at the doses tested (2000 mg/kg). Also no signs of apparent toxicity were observed.

In the literature, it is reported that plants with an LD₅₀ greater than 1000 mg/kg orally are considered non-toxic (Clarke and Clarke, 1977).



RESULTS AND DISCUSSION:

Acute Toxicity

- **Weight change of animals in different lots**

The data obtained for this parameter indicate that weight growth in all animals lots, reflecting their good physiological condition.

These informations suggests that the aqueous and hydro-ethanolic extracts of the ten plants studied show no apparent toxicity at the dose of 2000mg/kg.



RESULTS AND DISCUSSION:

Acute Toxicity

- Konan et al. (2007) and Jintanaporn et al. (2019) reported that at 2000 mg/kg, hydro-ethanolic extract from the leaves of *Anacardium occidentale* induced no mortality or signs of apparent toxicity.
- Same observation is by Ahmadu et al. (2003) for the ethanolic extract of *Daniella oliveri* and Ebbo et al. (2020) for the methanolic extract of the leaves of *Diospyros mespiliformis* but at a dose of 5000 mg/kg



RESULTS AND DISCUSSION:

Effect of the studied plant extracts on the biochemical parameters of rats.

- the plant extracts studied had no significant influence on the different biochemical parameters compared to the control group ($p > 0,05$).
- However, there was a significant decrease in ASAT levels in rats treated with the aqueous extract of *Ocimum gratissimum* ($p < 0.05$).



RESULTS AND DISCUSSION:

Effect of the studied plant extracts on hematological parameters

- All the extracts of the ten plants studied have no significant effect on these different hematological parameters of the animals ($p > 0.05$).



RESULTS AND DISCUSSION:

Histopathology study

- From kidney and liver tissues, histological sections were performed to confirm the haematological and biochemical data. For all extracts, histological sections of the organs of treated rats show no structural abnormalities compared to controls.



RESULTS AND DISCUSSION:

Histopathology study

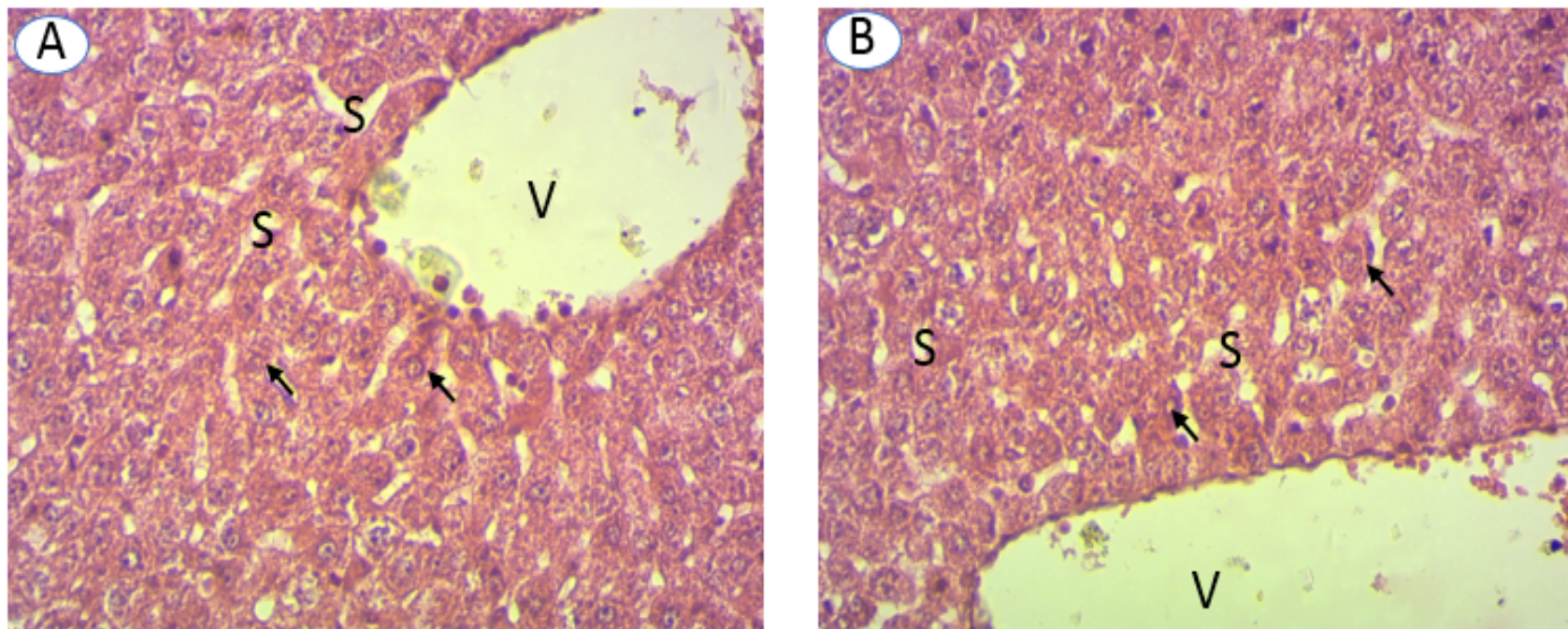


Figure 1: Hepatic histology of rats treated with extracts of the plants studied and control rats (A), 400x magnification



RESULTS AND DISCUSSION:

Histopathology study

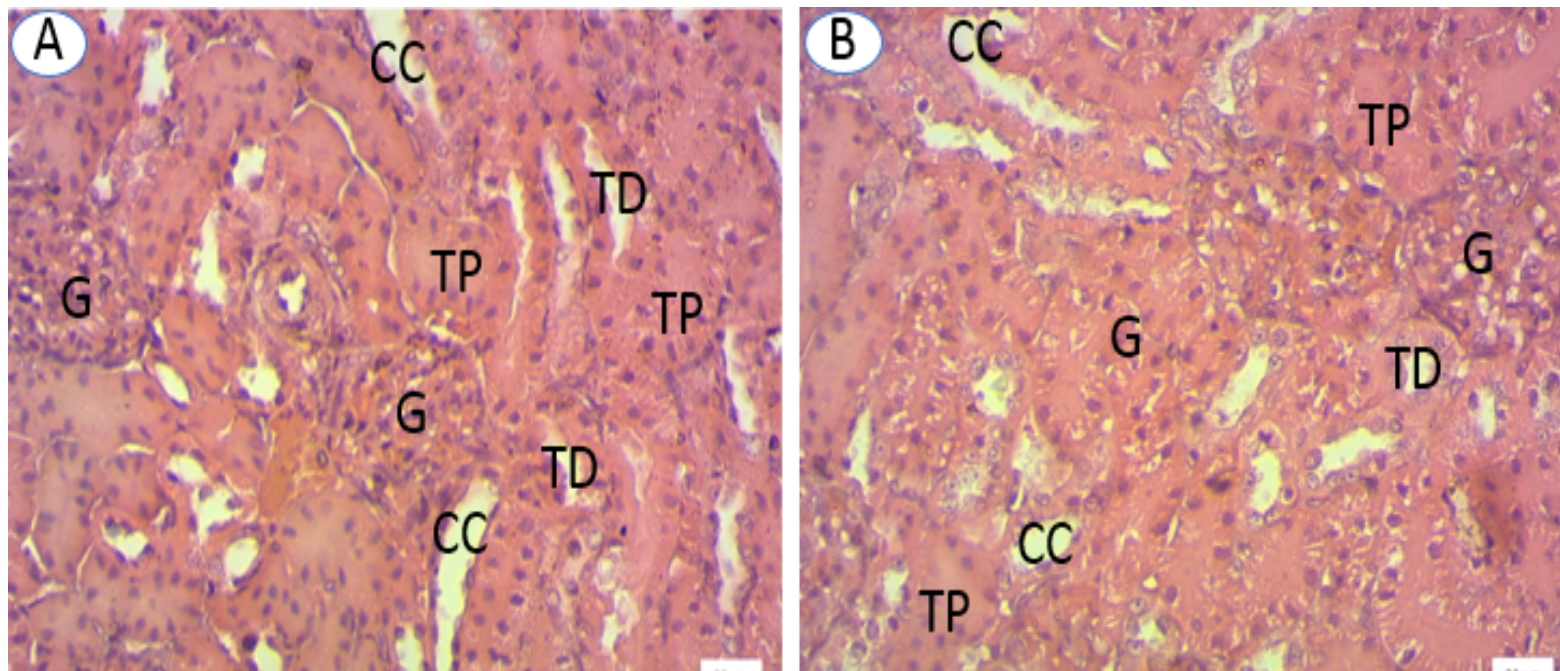


Figure 2: Kidney histology of rats treated with the plant extracts studied (Figure B) and control rats (A), 400x magnification



CONCLUSIONS

- The purpose of this study was to generate recent data on the phytochemical and toxicological characteristics of ten (10) plants used in the traditional treatment of diarrheal diseases.
- Phytochemical screening of the plants studied revealed a varied richness of secondary metabolites.
- These plants are not toxic according the data obtained. These results justify the use of plants in Beninese traditional medicine



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